

Antennal Proteins Involved in the Neural Mechanism of Quinone Inhibition of Insect Feeding

Findings about several aspects of the energy-transduction mechanism involved when certain quinones inhibit feeding in 2 species of insects were previously reported¹⁻¹⁰. These chemical messengers react with sulfhydryl groups of macromolecules in nerve membranes in chemosensitive sensilla especially on the antennae of the insects. Such reactions, as monitored by ultraviolet difference spectroscopy^{3,5}, change the conformation of macromolecules in isolated nerve-membrane fragments. This change in vivo presumably allows altered ion flow through the receptor membrane⁹, and this may bring about the generation of an action potential in the neuron¹¹. Because our previous studies indicated that proteins (i.e. especially sulfhydryl-containing moieties) were selectively involved in the receptor macromolecules, the binding affinity of the feeding inhibitor, 2-methyl-1, 4-naphthoquinone, for antennal proteins was further investigated. We now

present evidence of the resolution, by disc gel electrophoresis, of protein-containing bands from homogenized antennae of *Periplaneta americana* which appear to possess the properties of receptor chemical(s) for this feeding-inhibitory naphthoquinone. Results from in situ exposure of protein-containing materials in the antennae to 2-(¹⁴C) methyl-1, 4-naphthoquinone indicated that 2 closely associated bands in the electrophoretically resolved Triton X-100-soluble proteins contained relatively high amounts of label (Figure A) in contrast to a low degree of count associated with saline-soluble proteins (Figure B).

Materials and methods. Antennae of live cockroaches were held in a $2.4 \times 10^{-6} M$ aqueous solution of the radio-labelled naphthoquinone (9.7 mC/mM) for 0.5 h. The antennae then were rinsed extensively with tap water which degrades naphthoquinone on the surface of the antennae. The antennal proteins were extracted according

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to the methods of DAVIES¹². Treated antennae (100 mg wet wt/ml) were homogenized in 0.9% NaCl. The homogenate was centrifuged at 100,000 g for 60 min, and the pellet obtained was washed twice in the saline and centrifuged after each wash. The resultant supernatants were combined. The pellet was further extracted with 0.6% Triton X-100 in 0.9% NaCl by mechanical homogenization and ultrasonification. This latter extract was then centrifuged at 100,000 g for 60 min, and the supernatant was saved. To obtain solutions with a concentration of proteins suitable for analysis by disc gel electrophoresis, the saline and detergent extracts were concentrated by use of coarse Sephadex G25. The protein content of the concentrated solutions was determined according to LOWRY et al.¹³. The extracts were fractionated by disc gel electrophoresis as described by DAVIES¹². At pH 8.3, the proteins moved toward the anode. The gels were stained, sliced and prepared for liquid scintillation counting according to MUTO¹⁴. Each sample was counted at 95% efficiency for 20 min.

Results and discussion. Binding of the radiolabelled naphthoquinone to the saline-soluble proteins did not seem to indicate selective binding. In contrast, counts bound to certain detergent-soluble proteins did support selective complexing. The observed greater binding affinity of the quinone for Triton X-100-soluble than for saline-soluble proteins agrees with our previous findings⁵. Of the total counts applied in the solutions per replicate gel column (10,200 cpm per 200 µg saline-soluble protein and 80 µg TX-100-soluble protein) the percentage bound to the saline- and Triton-solubles was 1.6 and 4.5, respectively. The high binding capacity of certain detergent-extracted proteins becomes even more apparent when the amounts of bound label are compared on the basis of

quantity of protein applied per gel column. Though about 2.5 times more saline-soluble protein was run per column, the detergent-soluble proteins per column contained about 3 times as much bound radioactivity.

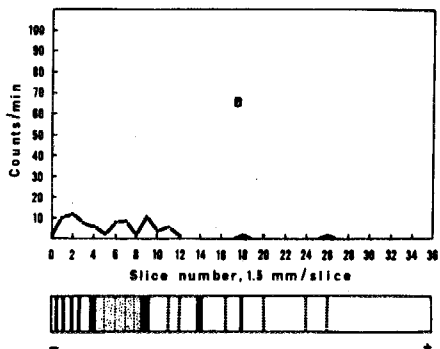
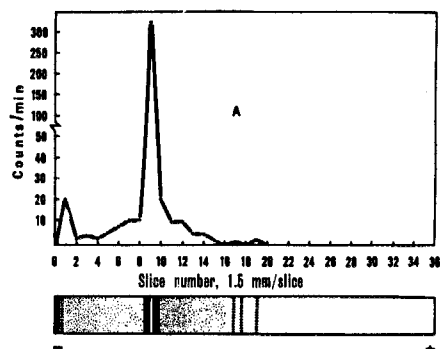
In this *in situ* study, physically accessible proteinaceous macromolecules which function naturally in chemoreception would be expected to especially interact with the quinone. From these results, we conclude that the 2 Triton X-100-soluble proteinaceous bands which selectively bound the quinone (Figure A) contain natural receptor sites for the feeding inhibitor.

Our various previous studies¹⁻¹⁰ of this energy transduction indicated that important initial *in vivo* energy transfer involves a basically reversible phenomenon. This characteristic seems especially reasonable in such primary interaction of relatively low concentrations of chemical messenger with sensory nerves. Under such conditions of reversibility, the degree of complexing of some concentration of messenger with receptor sites would be expected to reach equilibrium at some relatively low level per µg protein compared to levels with increasing degrees of irreversibility of complexing. In this regard, several types of meaningful reactions between natural messenger and groups of receptor sulphydryls of differing reactivities in neurons are apparently supported by our previous studies¹⁰. Some of the secondary interactions, associated especially with the more potent messengers, obviously are particularly characterized by less ready reversibility. The summation of complexing interactions of such varying reversibilities, upon extensive exposure of sensory neurons to messenger, probably is represented in our level of bound label in the double band of protein in Figure A¹⁵.

Zusammenfassung. Es wird gezeigt, dass der radioaktiv markierte Frasshemmstoff Methyl-naphthochinon in den Antennen von *Periplaneta* an 2 spezifische, mit Triton X-100 extrahierbare, elektrophoretisch trennbare Proteine gekoppelt wird.

S. M. FERKOVICH¹⁶ and D. M. NORRIS

Department of Entomology, University of Wisconsin,
Madison (Wisconsin 53706, USA), 27 December 1971.



Polyacrylamide gel electrophoresis- and bound radioactivity-patterns of Triton X-100-extracted (A) and saline-extracted (B) proteins from antennae (of living insects) held in an aqueous solution of 2-(¹⁴C) methyl-1,4-naphthoquinone ($2.4 \times 10^{-6} M$) for 0.5 h.

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- ¹⁶ Current address is USDA, ARS, Marked Quality Research Division, P.O. Box 14565, Gainesville (Florida 32601, USA).